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Heterogeneity is a characteristic feature of the process of intracellular protein catabolism. This holds true not only for considerable differences in the half-lives of cellular proteins, but it equally implies heterogeneity with regard to the mechanisms and pathways of intracellular protein breakdown (Hershko & Ciechanover, 1982). Experimental evidence shows that in many cells long-lived proteins are degraded mainly within lysosomes (Dean, 1980). In muscle cells, however, although they contain the bulk of long-lived myofibrillar proteins, only a small number of lysosomes are present (Berg & Bird, 1970). In this type of cell, therefore, non-lysosomal proteinases may play a more important role than in other non-muscle cells. As an example, several investigators have studied a 'myofibrillar' alkaline serine proteinase (for review see Bird et al., 1980), the activity of which is increased during several protein catabolic conditions (Dahlmann et al., 1984). This enzyme, however, originates from intramuscular mast cells (Stauber et al., 1983) and its importance for overall muscle protein breakdown may be questioned. Other than the mast cell-derived enzyme, an alkaline proteinase(s) exists in muscle tissue that shows elevated activity under hormone-induced catabolic conditions (Dahlmann & Reinauer, 1981).

To identify this enzyme(s), rat skeletal-muscle tissue was homogenized and extracted with 20 mM-Tris/HCl/1 mM-EDTA/1 mM-Na3/0.1% (v/v) 2-mercaptoethanol, pH 7.5. The extracted, soluble proteins precipitating at 45–65% saturation with (NH4)2SO4 were then chromatographed on a column of Sepharose 6B resulting in the resolution into four main peaks of proteolytic activity (Fig. 1).

Abbreviations used: Z, benzyloxy carbonyl; Suc, succinyl (3-carboxypropionyl); NMeC, 4-methyl-7-coumarylamide; Bz, benzoyl; NNap, 2-naphthylamide; NPhNO, nitroanilide.
must be at least \(4 \times 10^6\), suggesting that it is a complex of several proteins. The existence of a proteinase with similar size has been demonstrated in rat heart-muscle (DeMartino, 1983).

The proteinases eluting in the second peak hydrolysed the substrates \([^{14}C]\)methylcasein, Z-Phe-Arg-NMec, Suc-Ala-Ala-Phe-NMec and Bz-Val-Gly-Arg-NMec. These proteins were further fractionated by anion-exchange chromatography, resulting in the separation of three peaks with proteolytic activity (Fig. 2). The first peak, eluting at about 200 mM-NaCl, is a proteinase with a \(M_r\) of about 650 000 and it does not immunologically cross-react with the enzyme eluting in the second peak at 220 mM-NaCl (B. Dahlmann, unpublished work).

The latter enzyme, a cysteine proteinase with an \(M_r\) of about 750 000, hydrolyses the substrate Z-Phe-Arg-NMec optimally at pH 8–9 and is strongly inhibited by leupeptin, antipain and chymostatin (Dahlmann et al., 1983). The properties of this enzyme are very similar to those described for a high-\(M_r\) alkaline proteinase from rat heart (DeMartino, 1983) and immunological cross-reaction of both enzymes was recently observed in our laboratory (Dahlmann et al., 1983) and by DeMartino (1983), this proteinase is not inhibited by the microbial inhibitors leupeptin and chymostatin. These data suggest that more than one proteinase in skeletal-muscle tissue may be affected by ATP.

During the anion-exchange chromatography step a further enzyme, eluting at about 310 mM-NaCl from the resin, was identified (Fig. 2). This proteinase with an \(M_r\) of about 650 000 (Dahlmann et al., 1983) is composed of eight different species of subunits with \(M_r\) between 25 000 and 32000. The isoelectric point of the enzyme is at 5.1–5.2. This enzyme is designated 'multicatalytic proteinase' since it hydrolyses N-blocked tripeptide substrates with an arginine, phenylalanine or glutamic acid residue adjacent to the leaving group. Additionally, the enzyme contains caseinolytic activity and also degrades peptides like insulin, neurotensin and glucagons (Dahlmann et al., 1985a). As shown in Table 1, the activities towards tripeptide substrates and against \([^{14}C]\)methylcasein have different pH optima and they are affected by the microbial inhibitors leupeptin and chymostatin as well as by CaCl\(_2\) and KCl to a varying extent or even in an opposite fashion. This, together with the finding that the enzyme migrates as a single protein band in non-denaturing polyacrylamide-
gel electrophoresis as well as in isoelectric focusing gel and precipitates as a single arc in immunoelectrophoresis, indicates that these different activities reside within the same protein molecule and also suggests that the enzyme may contain more than one catalytic site (Dahlmann et al., 1985a).

Under normal physiological conditions the activity of a proteolytic enzyme must be under continuous control, e.g. by inhibitors, to prevent protein wasting by unlimited action of the protease. However, the modulation of a protease containing more than one active site may be difficult, especially when inactivation of one catalytic site is paralleled by activation of another catalytic site of the enzyme. This could be the reason for the observation that the multicalytic protease, when extracted from muscle tissue, is in a 'latent' state of activity. Upon addition to the system of high-M₉, alkaline proteinase is responsible for the increased (Dahlmann et al., 1981) and whether activities which probably resides in that protease molecule.

Further studies have to show whether the activity of this system of high-M₉, alkaline proteases is responsible for the enhanced non-mast cell derived alkaline autolytic activity in skeletal-muscle tissue as observed in insulin-dependent diabetic rats (Dahlmann & Reinauer, 1981) and whether these proteases are involved in the initiation of muscle-cell protein breakdown.

The physiological regulation of proteolysis in muscle

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Nutrition, hormonal regulation and amino acids

As recently emphasized (Millward et al., 1985) from the perspective of studies in vivo, proteolysis appears generally to be of less importance than protein synthesis in initiating nutritionally induced changes in protein balance (Millward et al., 1976; Rennie et al., 1983). Unlike the extreme liability of muscle proteolysis in vivo, in vivo it is better protected probably because of the regulatory importance of tension and membrane potential (see below). In our experience proteolysis only plays a leading role in initiating acute nutritionally mediated changes in muscle mass in unusual circumstances such as severe zinc deficiency (Giugliano & Millward, 1984; Millward et al., 1985).

We have previously argued that unlike tissues such as liver where amino acids play a major role in regulating protein balance and proteolysis (Poso et al., 1982), in muscle substrate supply is generally not involved (Millward & Waterlow, 1978). This is not to ignore the many studies showing the stimulatory effects of leucine on muscle protein synthesis and the inhibitory effect of its keto acid on proteolysis in incubated muscles (e.g. Mitch & Clark, 1984) but at least in the case of leucine the stimulation of protein synthesis in vivo has not been demonstrated (McNurlan et al., 1982). However, it is the case that in exercise when muscle proteolysis is reduced (as discussed below) plasma concentrations of the branched chain keto acids increase (Rennie et al., 1981).

The major known mediators of nutritional effects on muscle are insulin, corticosteroids and thyroid hormones, and all three have been implicated in the regulation of protein synthesis (Millward et al., 1983a) and proteolysis (Millward et al., 1985). Insulin and corticosteroids are jointly involved in the regulation of protein synthesis with insulin playing the major role (Millward et al., 1983b), but to the extent that these two hormones do regulate proteolysis in vivo, corticosteroids seem to play the dominant role (Millward et al., 1985).

In contrast there is a great deal of support for the importance of thyroid hormones (Millward et al., 1985). It would appear that the overall rate of proteolysis in muscle reflects the thyroid status at least as judged by the level of plasma free tri-iodothyronine (T₃). As shown in Fig. 1, when young rats are fed protein-deficient diets the changes in their plasma free T₃ (always accompanied by similar changes in their metabolic rate; Cox et al., 1984) are associated with similar changes in muscle proteolysis.

Abbreviations used: T₃, tri-iodothyronine; IL-1, interleukin 1; PG, prostaglandin; 3-MH, 3-methylhistidine.